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THE AUTORADIOGRAPHIC LOCALIZATION OF PARAQUAT IN THE LUNG

D. B. WEBB

From the K.R.U.F. Institute of Renal Disease, Royal Infirmary, Cardiff

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Summary.—Paraquat poisoning in mammals results in a characteristic lung lesion manifested principally as progressive pulmonary fibrosis. Paraquat is actively concentrated into the lung but the site of uptake remains undefined. A method is described for the autoradiographic localization of paraquat. Preliminary evidence for the site of uptake implicates the bronchiolar epithelium.

PARAQUAT (1,1'dimethyl,4,4'dipyridilium) is a major cause of mortality in human self-poisonings. Death either occurs acutely from multi-organ failure or may be delayed for days and follow progressive pulmonary fibrosis. Preceding the fibrosis there is loss of alveolar epithelium (Vijeyaratnam and Corrin, 1971) and an associated loss of surfactant (Manktelow, 1967). Paraquat is actively taken up into the lung (Rose, Smith and Wyatt, 1974) and this may predispose to pulmonary damage. The early damage to epithelial tissue and the existence of an active uptake mechanism has implicated the alveolar membrane as the site of uptake. Active uptake has also been shown in isolated pulmonary cell cultures (Schmitt and Autor, 1978) but the *in vivo* site of uptake remains uncertain. Although autoradiographic investigation should answer this problem, efforts so far have been unsuccessful. This paper describes a simple technique for autoradiography of the lung with ^{14}C-paraquat.

MATERIALS AND METHODS

Rats.—Adult female specific-pathogen-free Fisher inbred rats weighing 200–250 g were donated by the M.R.C. Pneumoconiosis Unit, Llandough Hospital, Penarth.

Paraquat.—(Methyl ^{14}C) paraquat, sp. act. 100 μC/mg (Radiochemicals, Amersham), was dissolved in 0·9% saline to give a dose of 1 μC/gm live weight in 2 ml. ^{12}C-paraquat dichloride (Imperial Chemical Industries, Central Toxicology Laboratory, Alderley Park) was likewise dissolved in 0·9% saline to the same concentration in 2 ml.

Injections and anaesthesia.—The rats were anaesthetized with i.p. chloral hydrate (36 mg/100 g live weight) for nephrectomy. Paraquat was injected i.p. No inhalation anaesthesia was used.

Autoradiographic development.—Sections were mounted on A.R. 10 stripping emulsion (Kodak Ltd, Hemel Hempstead) and developed in individual light tight containers at −70° for 14 weeks.

Experimental design.—Paraquat is rapidly excreted by the mammalian kidney unless renal failure secondary to paraquat intoxication supervenes (Davies, Hawksworth and Bennett, 1977). Accordingly when attempting autoradiography it is essential to induce renal failure or the small amount of isotopic paraquat will be rapidly excreted.

Renal failure was achieved by bilateral nephrectomy. Twenty-four h later ^{14}C-(methyl) paraquat was administered to one animal (250 μC total) and the same dose/g ^{12}C-paraquat to a second rat as a control. After a further 24 h the rats were killed by stunning, their chests opened at once and 3mm cubes of lung removed and snap-frozen in isopentane cooled in liquid N_2.

Autoradiographs were then prepared according to the technique of Appleton (1964) except that emulsion for preparing coated coverslips was floated out on fresh distilled water containing sucrose 20 g/l and KBr 0·01 g/l (Kodak Data Booklet).

RESULTS

The autoradiographs obtained are shown in Figs 1–4.

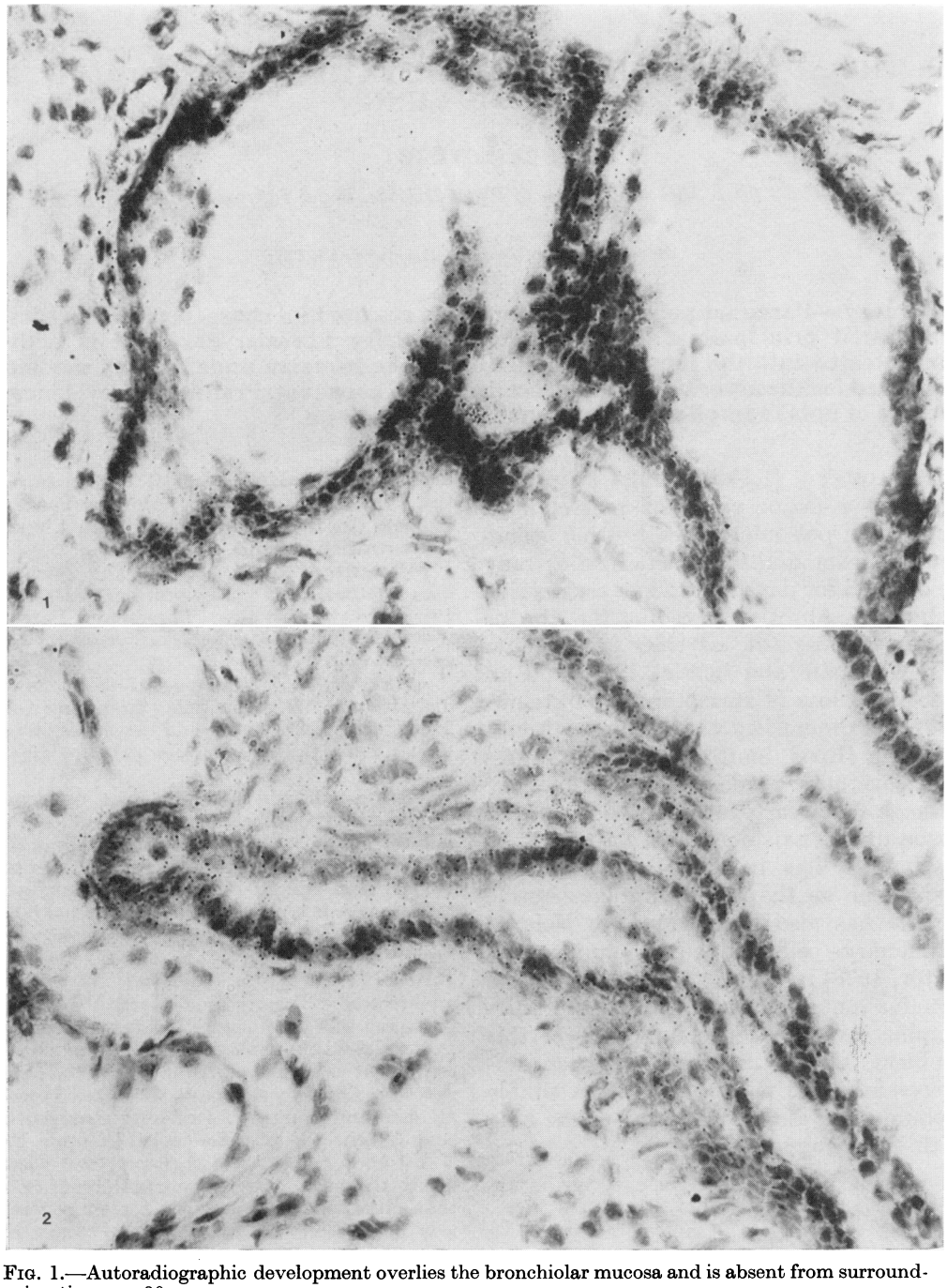

FIG. 1.—Autoradiographic development overlies the bronchiolar mucosa and is absent from surrounding tissue. × 80.

FIG. 2.—Autoradiographic development is most intense over the brochiolar mucosa. Some uptake is visible in adjacent tissue but not in alveoli (cf. Fig. 4). × 80.

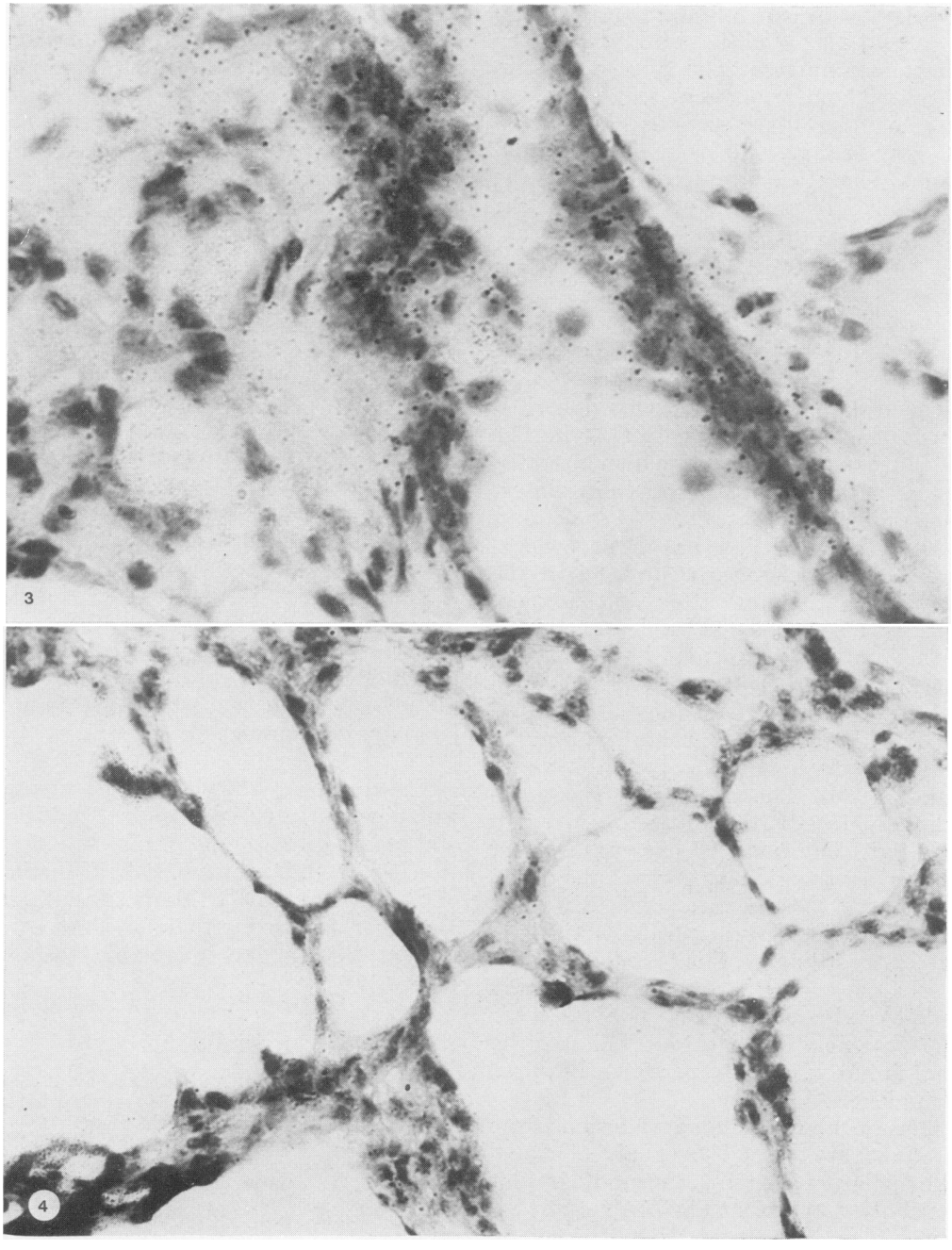

FIG. 3.—Higher magnification shows that autoradiographic development is principally related to the bronchiolar mucosa. × 128.

Fig. 4.—Autoradiograph: stippling is absent over the alveolar walls. × 80.

Distribution of autoradiographic development is almost confined to the bronchiolar mucosa. This is seen both in tangential and transverse sections. Over the remainder of the sections silver grains are very scanty. The control autoradiographs show no development of the emulsion. The presence or absence of the early changes of paraquat poisoning cannot be determined from these sections prepared from snap-frozen tissue.

DISCUSSION

The distribution demonstrated here is at the single time interval of 24 h. The relevance of the distribution demonstrated to that occurring in fatal poisonings must be examined.

Although attention has been concentrated on the changes that occur in the alveolar epithelium after intoxication, changes also occur in the bronchiolar epithelium (Vijeyaratnam and Corrin, 1971; Smith and Heath, 1974*a*,*b*; Etherton and Gresham, 1979). In the rat the LD_{50} of paraquat *via* the i.p. route is 19 mg/kg body wt (Clark, McElligott and Werbenblurst, 1966). The dose from the autoradiographic label amounts to 10 mg/kg body wt. Paraquat is excreted almost entirely *via* the kidneys (Daniel and Gage, 1966) and in these nephrectomized animals the circulating level will be artificially high. Accordingly this model mimics closely the clinical situation that usually leads to pulmonary disease (Davies *et al.*, 1977), namely intoxication followed by renal failure leading to persistently raised serum levels.

Thus, although pulmonary lesions from paraquat are spread over alveolar and bronchiolar epithelium, autoradiographic distribution appears to be confined to the bronchioles. This may relate to the increased mass of bronchiolar epithelium when compared to the flattened alveolar epithelium, thus permitting a greater mass of radioactive material to be apposed to the emulsion.

Pulmonary epithelium damaged by paraquat rapidly shows ultrastructural changes suggesting failure of metabolic function (Sykes, Purchase and Smith, 1977). If the alveolar epithelium rapidly takes up paraquat and is killed, the label will diffuse away. However, lung slices continue to take up paraquat at a time when such changes are evident (Rose and Smith, 1977) and, although patchiness of the lesions may permit this paradox, uptake and secretion by the bronchiolar mucosa into the bronchiole explains both this apparent contradiction and the autoradiographic distribution. Concentration of paraquat within the bronchiole will permit a rise in lung concentrations without a marked rise in intracellular concentration and destruction of the concentrating cells. Subsequently the epithelium around the bronchiole and alveolus will be damaged.

Further work is required to elucidate the time course of events and study ultrastructural changes simultaneously with autoradiographic localization. This technique provides a tool whereby this problem may be approached.

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